Comparative Study of Airborne Culturable Bacteria and Fungi in Lakes with and without Cyanobacterial Blooms

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Abstract:

Reports of increase respiratory illnesses and allergies are commonly associated with the presence of harmful algal blooms (HABs) in the area. A new method was developed to study if there is a relationship between HABs and other algal blooms and the presence of airborne fungi and bacteria. A remote control boat was used to drag two styrofoam boards with plates containing various selective media to culture bacteria aerosolized from the water. Lakes with and without HABs or other blooms were sampled. We report here on the diversity, quantity and composition of the airborne microbial flora collected in this comparative study. It is very likely that the health effects reported by people around HABs are the result of airborne microbes associated with the algal blooms, and not necessarily just the algae or the toxins alone. We demonstrated this is an effective method to collect culturable airborne microflora from lakes and ponds.

Introduction:

Harmful algal blooms (HABs) are becoming more popular across the world as over fertilization of crops continues and excess nitrogen leaks into streams, lakes, rivers, and eventually the ocean. HABs can occur in fresh and marine water and this is why it is important to understand the health risk associated with them before they become a bigger problem. HABs can come from any algae but cyanobacterial blooms are the largest suspects for the allergic reactions that are reported during the time of a HAB. Cyanobacteria can produce toxins and these toxins are specific to the species of cyanobacteria. Cyanobacteria are prokaryotic where as the rest of the algae are

eukaryotic. Cyanobacteria have also been on this planet for a very long time and they are primarily responsible for life on this planet because they produced all the oxygen and nitrogen gas present in our atmosphere through microbial processes.

Patients have been complaining about allergic reactions and respiratory illnesses during the event of a HAB (Torokne A, Palovics A, Bankine M. 2001), (Sharma NK, Rai AK 2006). This means that something is becoming airborne to make the people sick. This could be the toxin associated with cyanobacteria or it could be the bacteria and fungi present during the time of a HAB. When algal blooms develop other bacteria and fungi also grow because something has to eat the excessive biomass formed during a HAB. Bacteria and fungi have already been found to digest the toxins created by these algae and it is unlikely that the toxins are responsible for the allergic reactions because the cells must be lysed in order to extract the toxin (Bomo AM, Tryland I, Haande S, Hagman CH, Utkilen H. 2011). Unless the cyanobacterial cells are spontaneously combusting it is highly unlikely that toxin is the cause of these respiratory illnesses.

This is why it is important to determine if toxin or bacteria and fungi are present in the air during a HAB. This will aid doctors in better treatment techniques and it will allow them to understand why their patients are getting sick. The environmental protection agency (EPA) uses expensive membrane filtering techniques to sample the air but these were not available in this experiment. A simpler method had to be developed to test if larger bacterial and fungal counts were found in the air in lakes with HABs vs. lakes without HABs. This is the first step in determining if airborne bacteria and fungi are responsible for these respiratory like symptoms.

Materials and Methods:

A remote control boat was purchased from Toys R Us and used to tow two large Styrofoam boards that different selective and differential media was placed on. TSA, GYA, MSA, R2A, EMB, SAB, hektoen, and blood plates were placed on the boards- four on each Styrofoam mat. These plates were then drug around the lake with the remote control boat for a total of 12 minutes, brought back and then placed in a 37° C incubator for 2 days and then 27° C for 3 days. This was repeated for 3 lakes and this group was called the experimental group. The control was also repeated at the same 3 lakes and the same differential and selective plates were used. The control was not placed on the boat; instead these plates were placed in a position facing the part of the lake that had not been exposed to air that crossed the lake yet. This ensured that these plates would show what bacteria and fungi were already present in the air before crossing the lake. The control plates were placed in the same incubators for the same amount of time as the experimental group.

The results were quantified with the equation 5N/0.0069 where N= the number of colony forming units and this is multiplied by 5 because 5 * 12 = 60. The units for the final result are # cells/m^2/hr meaning this is a rate. This was done for all of the lakes for both groups and placed into an excel sheet. SPSS was used to analyze the statistical significance of the results and this is reported in the results section.

Results:

The data from excel was made into two bar graphs showing the amount of bacteria and fungi present for both the control and the experimental group and what was

left after the subtraction of the control. There was 4 times the amount of bacteria and fungi present in the lake with a cyanobacterial bloom than the two lakes without HABs. This was not found to be statistically significant because the sample size of three was not enough, and a larger sample size was needed to be statistically significant. The F-statistic was 4.025 and the p-value was .199.

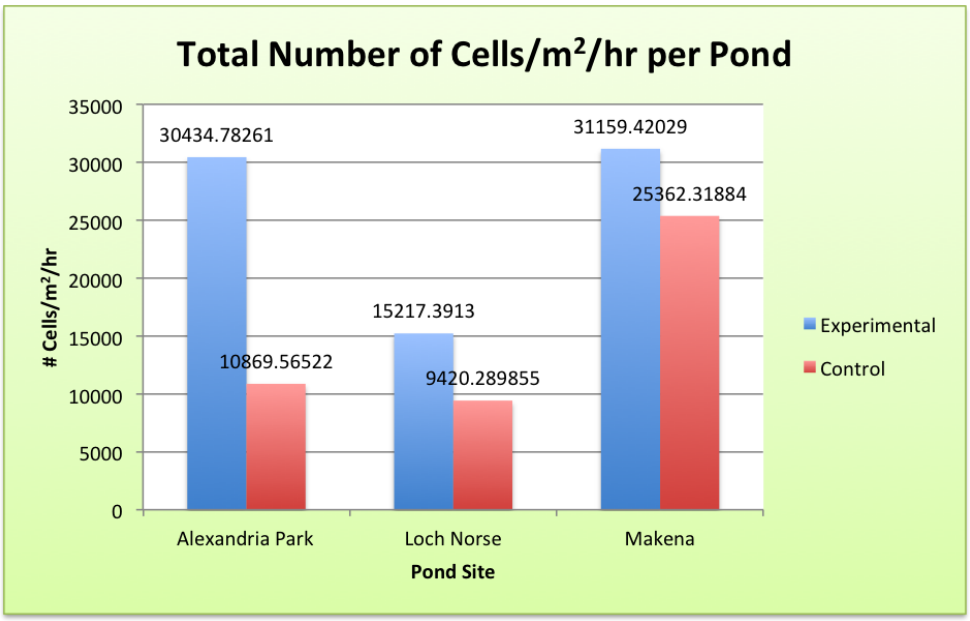

Figure 1. Figure one above is a bar graph displaying the total number of cells/m^2 that fell in one hour per pond.

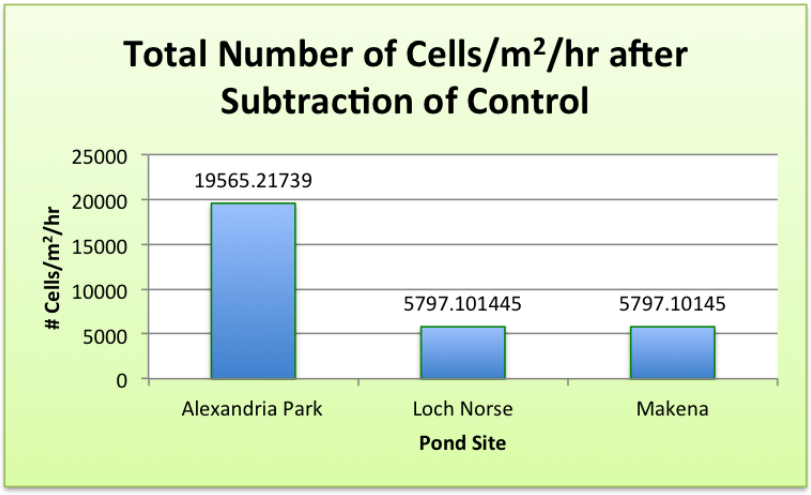

Figure 2. Figure two above is a bar graph displaying the number of cells/m^2/hr that was added to the lake after the control was subtracted from the total rate.

Randomized Block ANOVA

	Sum of Squares	Degrees of Freedom	Mean Square	F-statistic	P-value
Treatment	308.17	1	308.17	5.12	.152
Lake	484.33	2	242.17	4.025	.199
Error	120.33	2	60.17	X	X

Table 1. Table one above is a graph displaying the SPSS data.

Discussion:

Although SPSS did not agree that the results were statistically significant, the lake with cyanobacteria had 4X the amount of bacteria and fungi. This shows that there could be a direct link to HABs and a higher bacterial/fungal count in the air. The only reason SPSS did not show statistical significance is because the sample size was small. The sample size was small because the weather was not cooperating this spring and it wasn't warm enough for cyanobacterial blooms until April. The best time to sample lakes with cyanobacterial blooms in mid summer because lakes are no longer turning over and nutrients are on the top of lakes.

In the future this project should be conducted in mid summer and a sample size of 10 should be used to ensure statistical significance. This brings insight to the reason behind the allergic reactions and respiratory like illnesses experienced by patients exposed to lakes with HABs. Most importantly a new method has been developed to test this and it is far cheaper than the traditional methods of the EPA.

Bibliography:

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